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Toxicon 50 (2007) 292–301

TOXICON

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First report of homoanatoxin-a and associated dog neurotoxicosis in New Zealand

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Received 17 January 2007; received in revised form 27 March 2007; accepted 28 March 2007
Available online 13 April 2007

Abstract

In November 2005, at least five dogs died rapidly after contact with water from the Hutt River (lower North Island, New Zealand). Necropsy performed 24 h later on one of the dogs (a 20-month-old Labrador) revealed few findings of interest, except for copious amounts of froth in the respiratory tract down to the bifurcation of the trachea and large quantities of algal material in the dog's stomach. Low and relatively stable flows in the Hutt River during spring had resulted in the proliferation of benthic cyanobacteria that formed large black/brown mats along the river edge. Samples from the Labrador's stomach contents and cyanobacterial mats were analysed microscopically and screened using chemical and biochemical assays for cyanotoxins: anatoxin-a, homoanatoxin-a, cylindrospermopsins, saxitoxins and microcystins. Liquid chromatography–mass spectrometry (LC–MS) confirmed the presence of the neurotoxic cyanotoxins anatoxin-a and homoanatoxin-a and their degradation products, dihydro-anatoxin-a and dihydro-homoanatoxin-a. This is the first report of homoanatoxin-a and associated degradation product in New Zealand. Based on morphology, the causative species was identified as *Phormidium* sp. Subsequent phylogenetic analysis of 16S rRNA gene sequences demonstrated that the causative organism was most similar to *Phormidium autumnale*. Further investigations led to the detection of homoanatoxin-a and anatoxin-a in cyanobacterial mats from four other rivers in the Wellington region (lower North Island, New Zealand). Access restrictions were placed on over 60% of river catchments in the western Wellington region, severely affecting recreational users.

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Keywords: Homoanatoxin-a; Anatoxin-a; Benthic cyanobacteria; *Phormidium*; 16S rRNA; New Zealand

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1. Introduction

Cyanobacteria are known to produce cyanotoxins worldwide (Sivonen and Jones, 1999). The mechanisms of toxicity for cyanotoxins are very diverse, ranging from hepatotoxicity and neurotoxicity to

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0041-0101/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2007.03.025

dermatotoxicity. Some cyanotoxins have also been shown to promote liver tumour growth when ingested in low doses over extended periods (Falconer and Humpage, 1996; Kuiper-Goodman et al., 1999). Based on their chemical structure, cyanotoxins can be divided into the following three groups: cyclic peptides (microcystins and nodularins), alkaloids (cylindrospermopsins, anatoxins and saxitoxins) and lipopolysaccharides. Until recently, cyanotoxin production has primarily been associated with planktonic cyanobacteria. Although benthic taxa have received less attention than their planktonic counterparts, they are increasingly recognised as problematic and also produce a range of cyanotoxins (e.g. Edwards et al., 1992; Carmichael et al., 1997; James et al., 1997; Mez et al., 1997; Seifert et al., 2006).

Benthic, mat-forming cyanobacteria are widespread throughout the New Zealand rivers and found in a wide range of water quality conditions, including relatively un-enriched waters (Biggs and Kilroy, 2000). To date, the only documented poisoning incidences involving benthic cyanobacteria in New Zealand occurred when several dogs died after contact with the Waikanae River (1998) and Mataura River (1999). Samples collected from these rivers identified *Oscillatoria* sp. as dominant (Hamill, 2001). High-performance liquid chromatography analysis showed the presence of two dihydro-anatoxin-a isomers in the Waikanae River samples and toxicity in samples from both the rivers was confirmed by mouse bioassays with symptoms indicating anatoxin-a poisoning (Hamill, 2001).

The Hutt River (lower North Island, New Zealand) (Fig. 1) runs beside the heavily populated urban centers of Upper Hutt and Lower Hutt, before discharging into Wellington Harbour. In its northern reaches, the river is used to provide the cities of Wellington, Porirua and Upper and Lower Hutt with up to 40% of their potable water supply. The middle and lower reaches of the Hutt River are used extensively for recreational activities. Low and relatively stable flows in the Hutt River during spring 2005 resulted in the proliferation of benthic cyanobacteria that formed large black/brown mats along the river edge near Lower Hutt. In November and December 2005, at least five dogs died after contact with the river and cyanotoxin poisoning was suspected as the cause of fatalities. In this paper, we report on the screening for potential cyanotoxins in

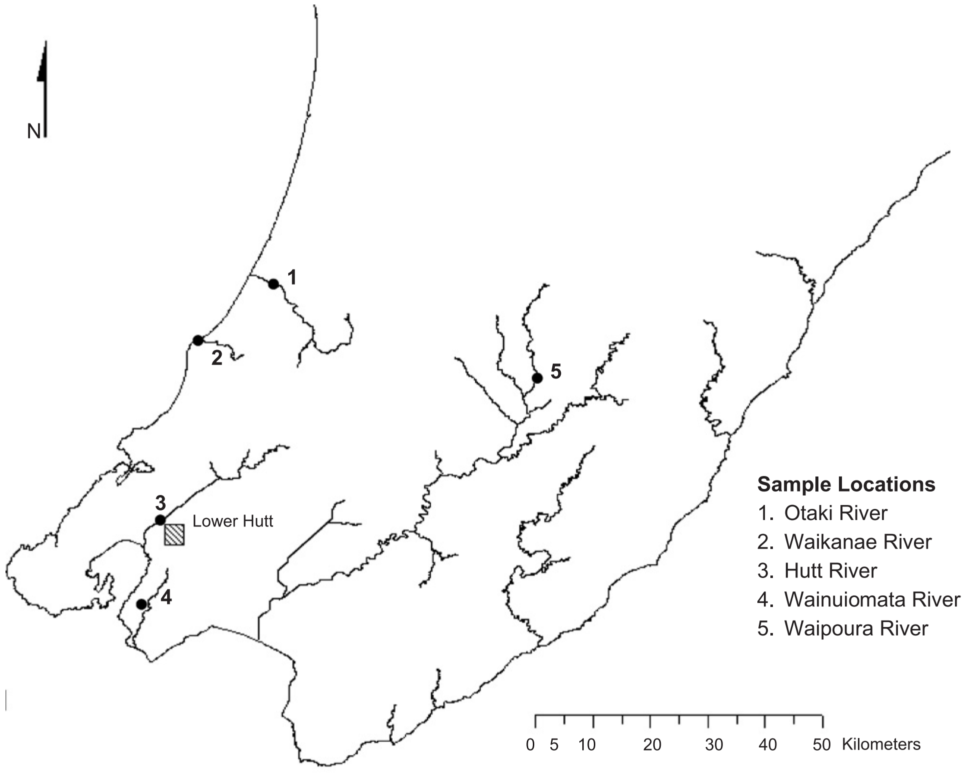

Fig. 1. Location of sampling sites, lower North Island, New Zealand.

cyanobacterial mats and from the stomach contents of one of the dead dogs. Additionally phylogenetic analysis based on 16S rRNA gene sequence was undertaken to identify the causative organism.

2. Materials and methods

2.1. Sample collection and site locations

Cyanobacterial mats were collected from the edge of the Hutt River (41°09′S; 174°59′E) on 20 November 2005 in close proximity to where dogs had been in contact with the river and subsequently died. Subsamples were preserved with Lugol's solution for morphological identification using light microscopy and the remainder frozen (−20 °C) for cyanotoxin and molecular analysis. Stomach samples from a dead dog (a 20-month-old Labrador) were received chilled on 22 November 2005. Subsamples were preserved with Lugol's solution for morphological identification and the remainder frozen (−20 °C) for cyanotoxin analysis. Samples of benthic mats from four other rivers within the Wellington region were collected between November 2005 and March 2006 and frozen for cyanotoxin analysis as described above. Samples collected were: Otaki River (24 November 2005, 40°45′S; 175°09′E), Waikanae River (23 November 2005, 40°53′S; 175°04′E), Wainuiomata River (14 December 2005, 41°16′S; 174°56′E) and the Waipoura River (13 March 2006, 40°57′S; 175°40′E) (Fig. 1). River flow and water temperature data for the Hutt River (collected at Taita Gorge, 41°09′S; 174°58′E) were provided by the Greater Wellington Regional Council.

2.2. Light microscopy of cyanobacterial mat and Labrador tissue

Morphological observations were made on the Lugol's preserved samples from the Hutt River using an Olympus BX51 microscope. The mean cell sizes of 30 cells were measured.

Samples of tissue fixed in 10% neutral-buffered formalin were received from the 20-month-old Labrador. The tissues were processed and embedded in paraffin blocks. Histologic sections were cut at 6 μm, mounted and stained with haematoxylin and eosin. Organs examined were heart, liver, kidney and lung.

2.3. Isolation of DNA

Subsamples of the frozen cyanobacterial mats from the Hutt River were lypholized. DNA was extracted from 0.1 g of lypholized material using the MoBio Power Soil™ kit (Carlsbad, CA, USA) according to the manufacturer's protocol.

2.4. Polymerase chain reaction (PCR)

PCR amplification of the 16S rRNA gene and the 16S–23S internal transcribed spacer (ITS) was performed in 25 μl reaction volume containing 30 ng of cyanobacterial mat DNA, 600 nM of each primer (338F 5′-ACT CCT ACG GGA GGC AGC-3′ and 23S30R 5′-CHT CGC CTC TGT GTG CCW AGG T-3′) (Invitrogen, New Zealand), 200 μM deoxynucleoside triphosphates (Roche Diagnostics, New Zealand), 1 × AmpliTaq PCR buffer (Roche Diagnostics, New Zealand), 0.75 U of AmpliTaq (Roche Diagnostics, New Zealand), 4 mM $MgCl_2$ (Roche Diagnostics, New Zealand) and 0.6 μg Bovine Serum Albumin (Invitrogen, New Zealand). PCR amplification employed a touchdown protocol to increase amplification specificity. Thermal cycling conditions were: 94 °C for 2 min followed by 94 °C 1 min; 65 °C 1 min, 72 °C 2 min for 19 cycles with the annealing temperature being gradually decreased by 0.5 °C per cycle, followed by 14 cycles of 94 °C 1 min, 55 °C 1 min, 72 °C 2 min and a final extension of 72 °C for 7 min. PCR reactions were run on a PTC-200 Peltier Thermal Cycler (MJ Research Inc., Massachusetts, USA).

2.5. Cloning and sequencing of ribosomal DNA

Cloning of the PCR product was performed with a TOPO TA cloning kit (Invitrogen, New Zealand) following the manufacturerer's instructions. Eight individual colonies of the clone library were randomly selected for plasmid recovery and analysis. The colonies were cultured in 30 ml of LB Broth (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract and 10 g l^{-1} NaCl) containing 50 μg ml^{-1} kanamycin at 37 °C for 16 h. Plasmids were extracted following the protocol described in Kotchoni et al. (2003) and the samples quantified with a NanoDrop (NanoDrop Technologies, Wilmington, USA).

The cloned ribosomal DNA was re-amplified with primer pair M13 according to the TOPO TA cloning kit protocol using 1 pg of plasmid template. Aliquots of 3 μl of amplification reactions were electrophoresed through a 1.5% agarose gel to verify the correct molecular size of cloned inserts. One representative PCR sample was purified with the GenScript DNA purification kit (GenScript Corp., USA) following the manufacture's instruction. DNA sequencing was carried out at the Waikato DNA Sequencing Facility using primers T3, T7 (TOPO TA cloning kit, Invitrogen, New Zealand) and 1392R (5′-ACG GGC GGT GTG TRC-3′) (Lane et al., 1985).

2.6. Phylogenetic analyses

The new 16S rRNA sequence was subjected to the taxonomical hierarchy of the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/) to determine its closest homologous relative. A phylogenetic tree was constructed with ClustalW 1.83 (European Bioinformatics Institute; http://www.ebi.ac.uk/clustalw/) using the neighbour-joining algorithm (Saitou and Nei, 1987) and the Kimura correction of branch distances (Kimura, 1890).

2.7. Cyanotoxin extraction and analysis

2.7.1. Anatoxins and cylindrospermopsins

Acetonitrile (5 and 1 ml) and formic acid (10 and 1 µl) were added to 5 g cyanobacterial mat sample and 1 g of dog stomach contents, respectively. Each sample was sonicated for 10 min. Following centrifugation (2000 *g*, 10 min), an aliquot of the supernatant was analysed directly by liquid chromatography–mass spectrometry (LC–MS). Hydrophilic toxins were separated by isocratic LC (Alliance 2695, Waters Corp., MA) using a 150 × 2 mm TSK gel Amide-80 5 µm column (Toyo-Haas, PA) with 95% mobile phase A (90% methanol) and 5% mobile phase B (112 mM formic acid plus 40 mM ammonia) at a flow of 0.2 ml min^{-1} (Dell'Aversano et al., 2004). Injection volume was 10 µl. The Quattro Ultima TSQ mass spectrometer (Waters-Micromass, Manchester) was operated in ESI^{+} mode with capillary voltage 2.5 kV, desolvation gas 500 l h^{-1}, 350 °C, cone gas 50 l h^{-1} and cone voltage 80 V. Quantitative analysis was by multiple reaction monitoring (MRM) using MS–MS channels setup for anatoxin-a (166.15 > 149.1; Rt 5.4 min), dihydro-anatoxin-a (168.15 > 91.15, Rt 5.3 min), homoanatoxin-a (180.2 > 163.15; Rt 4.9 min), dihydro-homoanatoxin-a (182.2 > 57.1, Rt 4.8 min), deoxycylindrospermopsin (400.3 > 194.15; Rt 6.5 min) and cylindrospermopsin (416.3 > 194.15; Rt 7.7 min). The instrument was calibrated with dilutions in 80% methanol of authentic standards of anatoxin-a (A.G. Scientific, CA) cylindrospermopsin and deoxycylindrospermopsin (National Research Council of Canada, Halifax, NS). Homoanatoxin concentrations were calculated using the anatoxin-a calibration curve. The profile and identity of anatoxins in concentrated cyanobacterial extracts were confirmed using full-scan LC–MS and liquid chromatography–tandem mass spectrometry (LC–MS/MS) with collisional activation spectra from selected [MH]$^{+}$ ions (collision energy 22 eV).

2.7.2. Microcystins

A subsample of the cyanobacterial mat was analysed by LC–MS/MS for the microcystin variants RR, diDMe-RR, DMe-RR, LR, YR, dMe-LR, FR, WR, AR, LA, LY, LW and LF and for nodularin. A 5 g subsample of the cyanobacterial mats was sonicated for 10 min with 5 ml of acetonitrile and centrifuged (3000 *g*, 10 min). An aliquot of the supernatant was taken for LC–MS analysis. Microcystins were separated by LC (Alliance 2695, Waters Corp., MA) using a 150 × 2 mm Luna C18 5 µm column (Phenomenex, CA) with water/methanol/acetonitrile gradient containing 0.15% formic acid (0.2 ml min^{-1}, 10 µl injection). The Quattro Ultima TSQ mass spectrometer (Waters-Micromass, Manchester) was operated in ESI^{+} mode with MRM using MS–MS channels setup for 13 microcystins and nodularin. The *m/z* 135 fragment from the protonated molecular cation was selected for each toxin (the doubly charged molecular species for MC-RR; the singly charged molecular species for all other toxins).

2.7.3. Saxitoxins

A subsample (0.5 g w/w) of cyanobacterial mat with 3 mM HCl added was sonicated for 10 min and centrifuged (3000 *g*, 5 min). The presence of saxitoxins was assessed in the extracts using the Jellett Rapid PSP Test kit (Jellett et al., 2002) according to the protocol supplied by the manufacturer.

3. Results

3.1. Morphology of Hutt River cyanobacterial mats

The Hutt River cyanobacterial mat strain showed the following characteristics: thallus olive-green to dark brown/black, thick, leathery and layered;

either attached to substrate or in free-floating mats on the river surface; trichomes olive-green to brown, 6–9 μm wide, straight or weakly curved, not constricted at the cross-wall and not attenuated at the ends; cells almost isodiametric, 4–9.5 μm long; apical cells obtuse rounded without calyptra (Fig. 2A). The cyanobacterial filaments and cells observed in the stomach of the 20-month-old Labrador had identical dimensions (Fig. 2B).

3.2. Genetic analysis

The partial sequence of the 16S rRNA gene (1171 bp) and the full ITS region of the Hutt River strain were cloned and sequenced (GenBank EF222209). RDP and NCBI analyses of the new ribosomal sequence matched with 98% sequence homology to *Phormidium autumnale* (GenBank DQ493873) with a sequence match score of 0.913, followed by *Tychonema bourrellyi*, 0.907 (GenBank AB045897) and *Oscillatoria* sp., 0.896 (GenBank AB074509). A phylogenetic tree was constructed using 16S rRNA sequences from a variety of Oscillatoriales. The Hutt River strain formed a subgroup with two *P. autumnale* strains (GenBank DQ493873 and DQ493874) (Fig. 3).

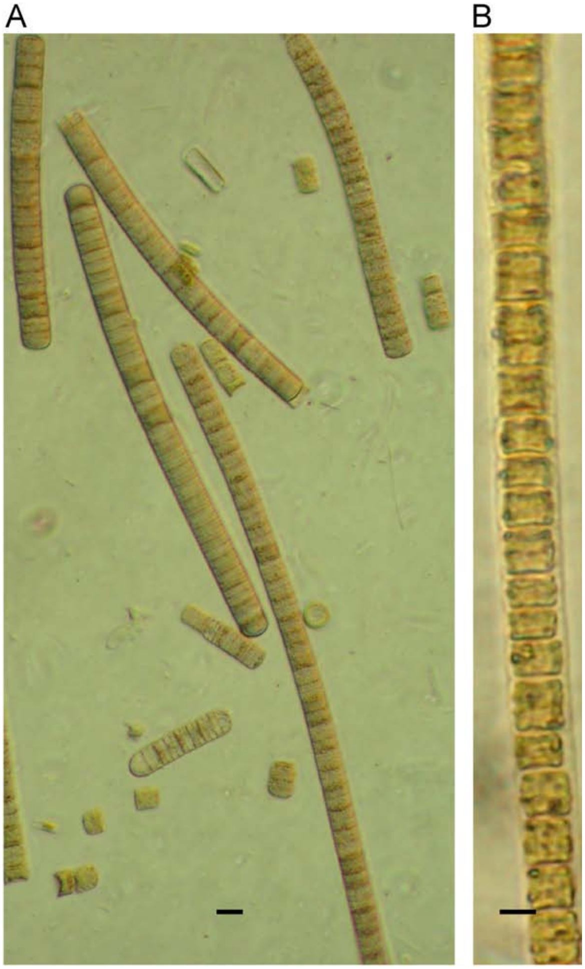

Fig. 2. Light photomicrographs of *Phormidium* sp. (A) Filaments from environmental samples. (B) Filament from dog stomach. Scale bars = 10 μm.

3.3. Cyanotoxin analysis

Cylindrospermopsin, deoxycylindrospermopsin and the 14 common microcystin variants were not detected (LODs ca 2 μg kg^{-1} for each toxin) in any sample using LC–MS analysis. Additionally, saxitoxins were not detected in any sample using a Jellett Rapid PSP test kit (LOD 3 μg kg^{-1}).

The sample collected from the Hutt River (20 November 2005) was positive for anatoxin-a and homoanatoxin-a (Fig. 4, Table 1). A very similar profile of anatoxin peaks was found for the sample from the Labrador's stomach contents (22 November, 2005). Dihydro-anatoxin-a and and dihydrohomoanatoxin-a were also detected. The peaks for these non-toxic metabolites were of similar or greater magnitude to those for the parent toxins but no standards were available for accurate quantitation. Samples collected from the four other rivers in the Wellington region were also positive for anatoxin-a and homoanatoxin-a, although at lower levels than in the Hutt River samples (Table 1).

Although the MRM analysis was very specific, interference by phenylalanine to the LC–MS analysis of anatoxin-a has been reported (Furey et al., 2005). This amino acid gave a very low response at a different retention time to anatoxin-a in our LC–MS/MS (MRM) analytical system. Characteristic peaks for phenylalanine and epoxy metabolites were not detected in full-scan LC–MS of the samples. Further confirmation of identity for the detected anatoxins was provided by LC–MS/MS spectra. The collisional activation spectra were very similar to the published spectra for anatoxin-a, homoanatoxin-a and their dihydro metabolites (Furey et al., 2003b; Dell'Aversano et al., 2004).

3.4. Pathology

The young dog was clinically healthy immediately prior to its death. A field post-mortem was conducted approximately 36 h after death. There

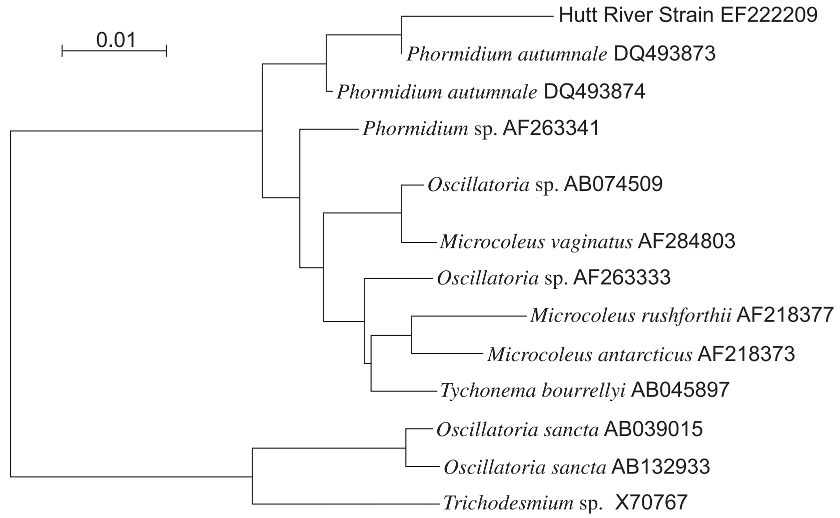
0.01
Hutt River Strain EF222209
Phormidium autumnale DQ493873
Phormidium autumnale DQ493874
Phormidium sp. AF263341
Oscillatoria sp. AB074509
Microcoleus vaginatus AF284803
Oscillatoria sp. AF263333
Microcoleus rushforthii AF218377
Microcoleus antarcticus AF218373
Tychonema bourrellyi AB045897
Oscillatoria sancta AB039015
Oscillatoria sancta AB132933
Trichodesmium sp. X70767

Fig. 3. Phylogenetic tree based on multiple sequence alignments (ClustalW) of 16S rRNA gene sequences of the Hutt River strain and selected Oscillatoriales. The scale bar denotes branch lengths. NCBI accession numbers are indicated after the species nomenclature.

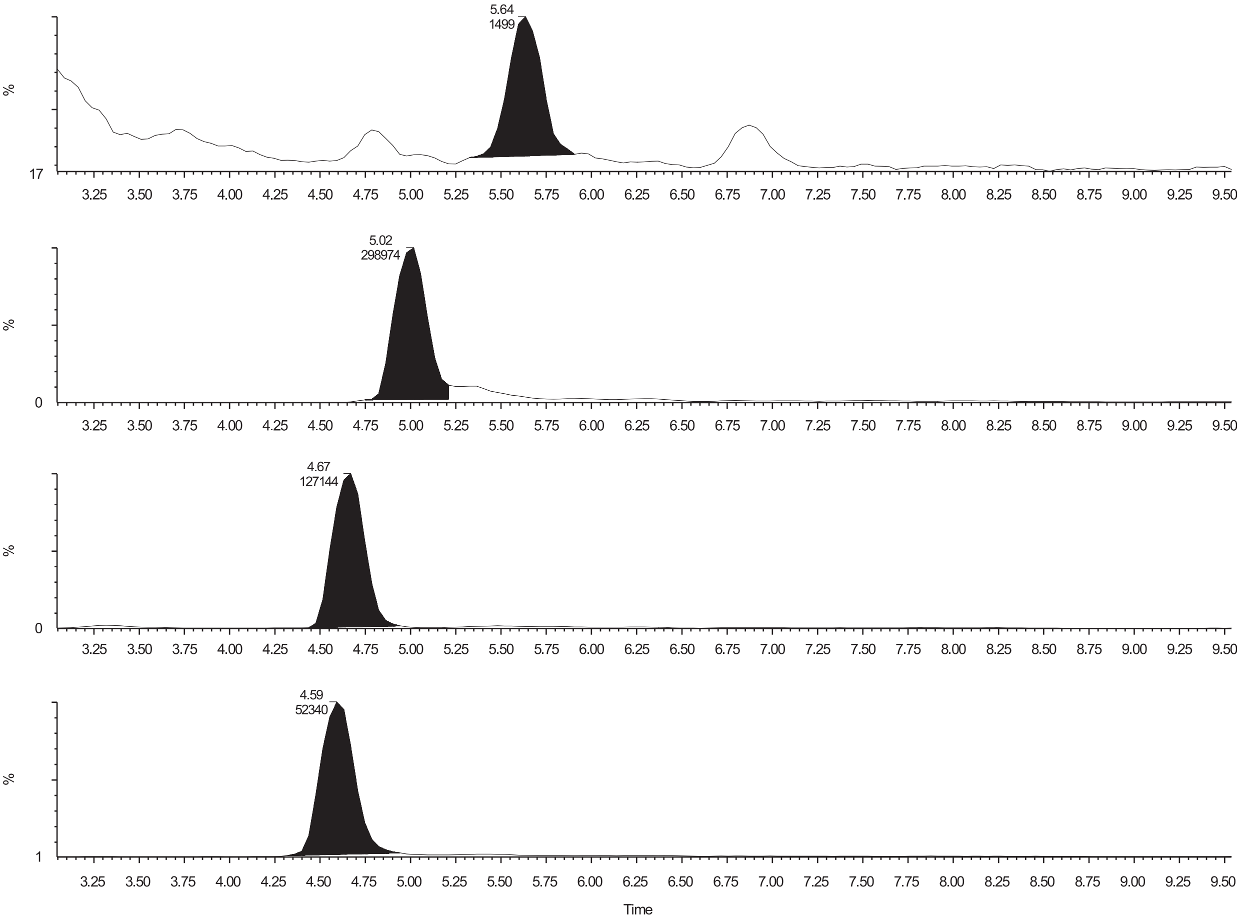

Fig. 4. LC–MS/MS MRM chromatograms for cyanobacterial mat from the Hutt River (20 November 2005). (A) Anatoxin-a, (B) dihydro-anatoxin-a, (C) homoanatoxin-a and (D) dihydro-homoanatoxin-a.

were no significant findings at necropsy except for copious amounts of cyanobacteria-contaminated water in the stomach and blood-tinged stable froth in the upper airways. Sections of lung floated in formalin. In histological sections, the lung had patchy flocculent eosinophilic fluid and some outlines of gas bubbles with sharp edges within the alveoli. There were no activated or haemosiderin-laden macrophages, no foreign material or dilated lymphatics seen in the lung. The liver and heart appeared normal. The kidney was congested and both liver and kidney were autolytic.

3.5. *Environmental conditions*

Analysis of hydrological and water quality data collected from routine monitoring by the Greater Wellington Regional Council during spring 2005 highlighted environmental conditions favourable to benthic cyanobacterial growth. Above average sunshine hours and air temperatures corresponded to increased water temperatures in the Hutt River at Taita Gorge (near the affected area) which were 1.4–2.6 °C above average for the August to November period (Milne and Watts, 2007). Spring rainfall was approximately half of the normal amount for the Hutt River catchment, and lower again for the Otaki River and Waikanae River catchments. The net result was below average flows in rivers throughout the Wellington region. During July to November 2005, the Hutt River at Taita Gorge was only above the median flow of 14 cumecs 29% of the time, compared to an average year in which it would be above the median flow 67% of the time (Milne and Watts, 2007). River flows were also relatively stable. Typically in spring, the Hutt River would experience three to four flushing flows (i.e. three times the median river flow) per month between July and November. However, in 2005, only one flushing flow was recorded in each of August and September, and no flushing flow was recorded in November (Milne and Watts, 2007) (Fig. 5).

Table 1
Cyanotoxin concentrations in cyanobacterial mat samples

Sample location	Date	μg/kg (wet weight)	
		Anatoxin	Homoanatoxin
Hutt River	20 November 2005	27	4400
Otaki River	24 November 2005	9.4	51
Waikanae River	23 November 2005	11	64
Wainuiomata River	14 December 2005	25	120
Waipoura River	13 March 2006	<0.5	110

4. Discussion

Recent surveys of toxic cyanobacteria in New Zealand have revealed their widespread and diverse distribution in New Zealand's water bodies (e.g. Wood et al., 2006a, b, 2007). These studies have primarily focused on toxin production by planktonic cyanobacteria and identified microcystins, anatoxin-a, cylindrospermopsins, nodularin and saxitoxins. Wood et al. (2006a) and Hamil (2001)

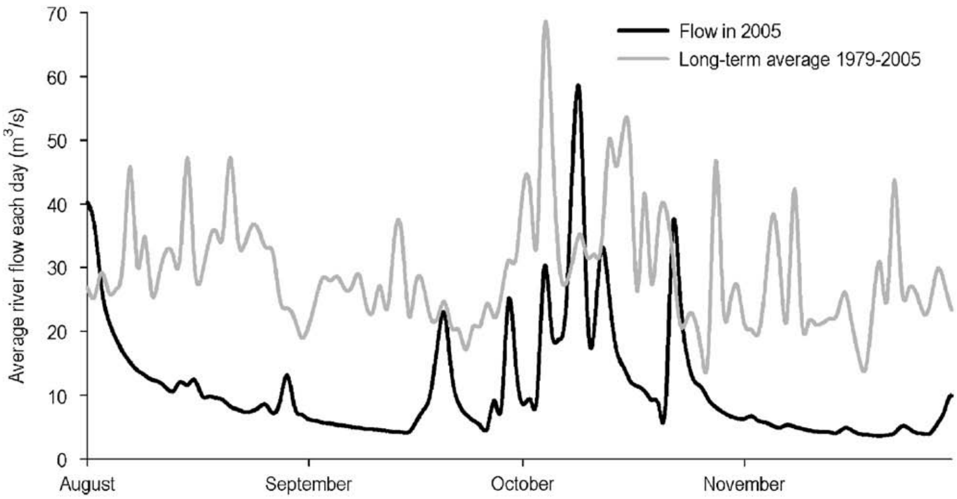

Fig. 5. Average daily river flows in the Hutt River over August to November 2005 and the long-term average daily flow (1979–2005).

detected microcystins in benthic cyanobacterial mats, and bioassays have indicated the presence of neurotoxins in *Oscillatoria* sp. mats (Hamil, 2001). The present study is the first report of homoanatoxin-a from cyanobacteria and poisoned-animal samples in New Zealand. In addition, it provides confirmation of the production of anatoxin-a by benthic species in New Zealand. The detection of anatoxin-a and homoanatoxin-a in both the cyanobacterial mats and dog's stomach contents, coupled with the presence of cyanobacterial filaments in the stomach and death of the dog in the absence of other causes, provides strong evidence that the two neurotoxins caused the sudden death of the 20-month-old Labrador. Histological examination of the dog's tissues was limited, but no underlying pre-existing health problem was found, nor any evidence of inflammation or infection. The lung changes of pulmonary oedema were consistent of agonal inspiratory gasping and shock (Dungworth, 1993). The other organs, although autolytic, did not show any abnormality. Drowning was ruled out due to the time periods elapsing between swimming and death (1 h) and healthy appearance of the animal on the walk home. The dog was found dead, so clinical signs of the dying animal were not observed. However, other dogs that died (no autopsies undertaken) after contact with the Hutt River in November 2005 were reported to shake, convulse, froth at the mouth and collapse shortly before death. These are all symptoms commonly associated with anatoxin-a or homoanatoxin-a poisoning (Ressom et al., 1994).

To date, homoanatoxin-a producing cyanobacteria have been found solely in the Northern Hemisphere and restricted to a few genera, including *Raphidiopsis mediterranea* in Japan (Namikoshi et al., 2003), *Oscillatoria formosa* in Sweden (Skulberg et al., 1992), *Oscillatoria* spp. from an unknown location (Araoz et al., 2005) and in environmental samples from Ireland containing predominantly *Anabaena* spp. (Furey et al., 2003a). We have recently isolated a cyanobacterium, *Aphanizomenon issatschenkoi*, from a New Zealand lake that produces anatoxin-a (Wood et al., 2007; Selwood et al., 2007). However, this planktonic species does not produce homoanatoxin-a. This work is therefore the first confirmation of a homoanatoxin-a-producing species in the Southern Hemisphere and the first report of homoanatoxin-a production by *Phormidium* sp. The identification of homoanatoxin-a in *Phormidium* sp. is not surprising as previously species from this genus have been shown to produce a range of cyanotoxins. *Phormidium favosum* isolated from a river in France produced anatoxin-a (Gugger et al., 2005), and an unidentified toxin with protracted effects in mice bioassays was found in *P*. aff. *formosum* and *P*. aff. *amoenum* surface mats from Australian water reservoirs (Baker et al., 2001). Recently, Teneva et al. (2005) investigated the toxin potential of five *Phormidium* spp. (*P. bijugatum*, *P. molle*, *P. papyraceum*, *P. uncinatum*, *P. autumnale*) with all strains causing neuro/hepatotoxic symptoms in mice.

Morphological and molecular investigation in this study demonstrated that the causative species was most likely *P. autumnale*. The morphological description of the Hutt River strain conforms to species descriptions of *P. autumnale* by Komárek and Anagnostidis (2005). However, thickened outer walls or calyptras at apex were not visible. Komárek and Anagnostidis (2005) note that these are not commonly seen in environmental samples or cultures. Identification of species based solely on morphological criteria is not always reliable because they may change due to environmental factors. For this reason, phylogenetic analysis based on the 16S rRNA gene sequences from the Hutt River strain was undertaken, indicating its closest homologous relatives were two *P. autumnale* strains (GenBank DQ493873 and DQ493874). Phylogeny also revealed a close evolutionary association of the Hutt River strain towards *T. bourrellyi* (GenBank AB045897). However, the morphology and biotype of the Hutt River strain does not conform to that of *T. bourrellyi* as described in Komárek and Anagnostidis (2005). Thorough morphological and molecular investigations of the mat-forming cyanobacteria species in the other rivers in the Wellington region were not undertaken; however, routine sample analysis (based on morphology) identified these as *Phormidium* spp. In these specimens, elongated, truncated calyptra were commonly observed, a frequent feature of *Phormidium* spp.

Phormidium spp. are common and cosmopolitan. Extensive attached or detached mats have been documented in many countries. In the Wellington region alone, *Phormidium* spp. were identified at 30 out of 46 long-term monitoring river and stream sites over the 2005 and 2006 summer (Greater Wellington Regional Council, unpublished data). The extensive proliferation of *Phormidium* spp. mats in the Hutt River in late 2005 was most probably associated with the exceptionally low and stable flows and elevated water temperatures during the spring of 2005. The detection of anatoxin-a and homoanatoxin-a in four other rivers in the Wellington region and previous incidences of unexpected dog, bird and stock deaths (Hamil, 2001) indicates that these cyanotoxins may be widespread and pose an underrated health risk to animals and humans in New Zealand riverine environments. Unusual environmental conditions such as those observed in Wellington during spring 2005 may act as indicators of impending benthic cyanobacterial proliferations.

There is a growing awareness among water users of the health risks associated with toxic planktonic cyanobacteria; however, the dangers associated with benthic cyanobacteria are less widely acknowledged. Currently, there is a lack of national guidance and policy for tackling the complex issues associated with their monitoring and management. In New Zealand, substantial guidance is given in the "Drinking-Water Standards for New Zealand 2005" and "Guidelines for Drinking-Water Quality Management for New Zealand 2005" (Ministry of Health, 2005a, b) on how to prevent, detect and manage cyanobacterial bloom events in the drinking water sources. Similarly, various documents used by managers of recreational water bodies provide cell concentration-based alert levels and practical advice on planktonic cyanobacterial monitoring and management. These documents however provide no information on benthic cyanobacteria. Existing guidelines for detecting and managing planktonic cyanobacteria in standing waters cannot be transferred directly to benthic taxa in flowing waters, due to fundamental differences in their biological requirements and the environmental niches that they inhabit.

The occurrence of toxin-producing cyanobacteria in rivers in the Wellington region in 2005/2006 resulted in public health warnings being issued on over 60% of the river catchments in the western part of the region, severely hindering recreational activities in those catchments. In addition, low flows and the persistence of mat proliferations in the Waikanae River led to the local council's decision to switch its potable water supply from river water to bore water. Healthy mats may pose little risk as the majority of cyanotoxins are likely to be intracellular and thus not released into the water supply. However, if the mats die or detach from their substrate, they may release massive pulses of cyanotoxins into a water supply intake. Currently, sampling techniques, e.g. taking grab samples of water near the intake, would miss these pulses and thus significantly underestimate the risk posed by benthic cyanobacteria. Further research is required in New Zealand to establish the extent and latent risks posed by benthic cyanobacteria, particularly in drinking water supplies.

Acknowledgements

The authors would like to thank Howard Markland, Tim Strange and Alton Perrie (Greater Wellington Regional Council) for the assistance with sample collection. S Wood thanks the New Zealand Foundation for Research, Science and Technology for post-doctoral fellowship funding (CAWX0501).

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